Anal Bioanal Chem
DOI 10.1007/s00216-006-0756-z

ORIGINAL PAPER

Application of double-spike isotope dilution for the accurate determination of Cr(III), Cr(VI) and total Cr in yeast

Lu Yang · Elena Ciceri · Zoltán Mester · Ralph E. Sturgeon

Received: 19 June 2006 / Revised: 10 August 2006 / Accepted: 10 August 2006

Abstract A method is presented for the simultaneous determination of Cr(III) and Cr(VI) in yeast using species-specific double-spike isotope dilution (SSDSID) with anion-exchange liquid chromatography (LC) separation and sector field inductively coupled plasma mass spectrometric (SF-ICP-MS) detection. Total Cr is quantitated using ID SF-ICP-MS. Samples were digested on a hot plate at 95±2 °C for 6 h in an alkaline solution of 0.5 M NaOH and 0.28 M Na_2CO_3 for the determination of Cr(III) and Cr(VI), whereas microwave-assisted decomposition with HNO_3 and H_2O_2 was used for the determination of total Cr. Concentrations of 2,014±16, 1,952±103 and 76±48 mg kg^{-1} (one standard deviation, n=4, 3, 3), respectively were obtained for total Cr, Cr(III) and Cr(VI) in the yeast sample. Significant oxidation of Cr(III) to Cr(VI) (24.2±7.6% Cr (III) oxidized, n=3) and reduction of Cr(VI) to Cr(III) (37.6±6.5% Cr(VI) reduced, n=3) occurred during alkaline extraction and subsequent chromatographic separation at pH 7. Despite this significant bidirectional redox transformation, quantitative recoveries for both Cr(III) and Cr(VI) were achieved using the SSDSID method. In addition, mass balance between total Cr and the sum of Cr(III) and Cr(VI) concentrations was achieved. Method detection limits of 0.3, 2 and 30 mg kg^{-1} were obtained for total Cr, Cr(VI) and Cr(III), respectively, based on a 0.2-g sub-sample.

L. Yang (✉) · Z. Mester · R. E. Sturgeon
Institute for National Measurement Standards,
National Research Council Canada,
Ottawa, Ontario K1A 0R6, Canada
e-mail: Lu.Yang@nrc-cnrc.gc.ca

E. Ciceri
Department of Chemical and Environmental Sciences,
University of Insubria,
Via Valleggio,
Como, Italy

Keywords Cr(III) · Cr(VI) · Double-spike isotope dilution · Yeast · ICP-MS

Introduction

Chromium has been extensively studied in the fields of environmental science, toxicology, and nutritional and analytical sciences. Amongst the various oxidation states possible (i.e. II, III, IV, V and VI), Cr(III) and Cr(VI) are the most significant, only form in aqueous solutions and possess very different toxicities. Chromium(VI) is readily absorbed by the lungs, digestive tract, mucous membranes and skin and is toxic and carcinogenic to humans and other animals [1–4]. On the other hand, Cr(III) is an essential nutrient to humans at the trace level and is required for normal carbohydrate and fat metabolism [1–4] with intake derived mainly from foods. Insufficient dietary intake of chromium leads to increased blood sugar, triglycerides and cholesterol levels, increasing the risk for diabetes and heart disease. Chromium deficiency occurs in elderly individuals, diabetics and those consuming large amounts of carbohydrates and sugars. Studies [5–10] have shown that chromium supplements can help with many conditions, including reducing blood sugar levels as well as the amount of insulin needed by diabetics, lowing cholesterol levels in the blood, improving lean body mass and reducing body fat for weight loss. As a result, consumption of Cr supplements has become popular, with chromium-enriched yeast being preferred. Because the nutritional bioavailability and toxicity of Cr are highly dependent on its chemical form and concentration, speciation of Cr in such supplements, especially the determination of Cr(VI), is of paramount of importance in safeguarding consumer health.

Speciation of Cr(VI) and Cr(III) is a most challenging task for analytical chemists due to the complex chemistry involved [11–15]. The relative amounts of Cr(VI) and Cr(III) in a sample depend on the electrochemical potential of redox reactions and ambient pH, which may be influenced by the presence of oxidizing and reducing agents in the sample matrix. Cr(III) is stable at low pH, whereas high pH favours Cr(VI). Inter-conversions between Cr(III) and Cr(VI) in environmental samples have been frequently observed during sample storage, extraction and measurement processes [11–15], leading to inaccurate speciation data when using methods which are incapable of correcting for these effects. For this reason, in 1986 the US Environmental Protection Agency (US EPA) withdrew method 3060, a procedure for determination of Cr(VI) in soils [11, 16]. Recent studies by Kingston's group [11–15] have solved this problem by using species-specific isotope dilution mass spectrometry (SSID MS) for the highly accurate determination of Cr(VI) in environmental samples that cannot otherwise be determined using conventional instrumental analytical methods. A double-spiking approach permits accurate corrections to be made for redox species transformations that may occur during sample processing. This SSID MS method has subsequently been adopted by the US EPA as method 6800 for the accurate determination of Cr(VI) in solid environmental samples [17].

To date, only quadrupole-based inductively coupled plasma mass spectrometry (qICP-MS) has been used for such studies [11–15] with a focus on only Cr(VI). In addition, no mass balance verification between the measured concentration of total Cr and the sum of concentrations for independently measured Cr(III) and Cr(VI) in the sample has been undertaken. Detection of chromium by qICP-MS is often complicated by polyatomic interferences, including $^{38}Ar^{12}C^{+}$, $^{36}Ar^{14}N^{+}$, $^{40}Ar^{12}C^{+}$, $^{35}Cl^{16}O^{1}H^{+}$, $^{37}Cl^{16}O^{+}$, $^{40}Ar^{12}C^{+}$, $^{40}Ar^{13}C^{+}$ and $^{40}Ar^{12}C^{1}H^{+}$ arising from constituents in the sample matrix [18]. High mass resolution sector field ICP-MS (SF-ICP-MS) can separate these interferences from the Cr isotopes, thereby providing an accurate measure of their intensities. In addition to its high resolution and high sensitivity (in low resolution mode), SF-ICP-MS typically yields more accurate and precise isotope ratio measurements compared to qICP-MS [19], thereby providing enhanced analytical precision.

Despite the dramatically different toxicities of Cr(III) and Cr(VI) and the increasing use of Cr supplements, accurate determination of these species in such supplements is lacking. Only one publication [20] targeting speciation of Cr (in chromium picolinate supplements) was conducted with use of LC ICP-MS for detection but without application of a double-spike ID methodology. The objective of this study was to take advantage of the intrinsic accuracy offered by the SSID MS approach when combined with an LC separation of the species and enhanced precision of measurement offered by SF-ICP-MS for the accurate and precise simultaneous determination of Cr(III) and Cr(VI) in a yeast supplement. Total Cr was also measured by SF-ICP-MS using isotope dilution and reverse isotope dilution to verify mass balance. Enriched ^{50}Cr(III) and ^{53}Cr(VI) spikes were used for the redox speciation of Cr. A reverse spike isotope dilution approach was performed to quantify the concentrations of enriched ^{50}Cr (III) and ^{53}Cr(VI) in the spikes. This appears to be the first report of the application of SSID-LC-SF-ICP-MS for speciation of Cr in dietary supplements.

Experimental

Instrumentation

A ThermoFinnigan Element2 sector field ICP-MS instrument (Bremen, Germany) equipped with a Scott-type double-pass glass spray chamber and a glass self-aspirating nebulizer was used. Optimization of the Element2 was performed as recommended by the manufacturer. Detector dead time was determined by following the procedure of Nelms et al. [21] (method 2) whereby a plot of $^{238}U/^{235}U$ ratio versus U concentration was constructed using solution concentrations of 0.5, 1.0 and 2.5 ng mL^{-1}, from which a dead time of 16 ns was derived.

A Dionex HPLC Model AGP-1 (Dionex, Sunnyvale, CA) and an anion-exchange column (Dionex, Omnipac PAX-500, 4.6 mm×250 mm) were used for separation of Cr species. A 0.06 M nitric acid/ammonium hydroxide solution at pH 7.0 was used as the mobile phase at a flow rate of 1.00 mL min^{-1}. Samples were injected onto the column using a microinjection valve from a Dionex Model LCM liquid chromatography module, equipped with an injection loop of nominal 100-μL volume. The coupling of HPLC to the Element2 was accomplished by directing the eluate from the column to the nebulizer through a 0.3-m length of PEEK tubing (0.254-mm i.d., 1.59-mm o.d.) connected to sample uptake tubing by a 2-cm length of a PVC tubing (0.76-mm i.d.).

Reagents and solutions

Nitric and hydrochloric acids were purified in-house prior to use by sub-boiling distillation of reagent-grade feedstock in a quartz still. Environmental-grade ammonium hydroxide and sodium carbonate were purchased from Anachemia Science (Montreal, Quebec, Canada). Double sub-boiling-distilled $HClO_4$ was purchased from Seastar Chemicals (Sydney, BC, Canada). High-purity de-ionized water (DIW) was obtained from a NanoPure mixed bed ion-exchange system

fed with reverse osmosis domestic feed water (Barnstead/Thermolyne Corp, Iowa, USA). A 2.5 M solution of NaOH was prepared by dissolving 100 g NaOH (Certified ACS, Fisher Scientific, Nepean, Ontario, Canada) in 1,000 mL DIW. A 0.06 M nitric acid/ammonium hydroxide eluent solution was prepared by adding 3.9 mL concentrated HNO_3 to 1 L DIW and adjusting the pH to approximately 7.0 with ammonium hydroxide. A 2,000 mg kg^{-1} stock solution of natural abundance Cr [as Cr(III)] was prepared by dissolution of the high-purity metal (Johnson, Matthey & Co. Limited, London, UK) in HCl. A working standard of 112 mg kg^{-1}, used for reverse spike isotope dilution of total ^{50}Cr, $^{50}Cr(III)$ and ^{53}Cr (VI), was prepared by dilution of the stock with DIW containing 1% HNO_3. A 1,000 mg kg^{-1} natural abundance Cr (VI) stock solution was prepared by dissolution of $K_2Cr_2O_7$ (Certified ACS, Fisher Scientific, Nepean, Ontario, Canada) in DIW.

Enriched ^{50}Cr isotope spike (97% +) as metal and ^{53}Cr isotope spike (97% +) as Cr_2O_3 were purchased from Trace Sciences International Corp (Richmond Hill, ON, Canada) and used to prepare ^{50}Cr, $^{50}Cr(III)$ and ^{53}Cr (VI) spike solutions, respectively. The enriched spike stock preparation was based on the procedure reported in US EPA method 6800 [17]. A ^{50}Cr stock solution of ca. 350 mg kg^{-1} was prepared by dissolution of the metal in a few millilitres of HCl followed by dilution with 2% HNO_3. A $^{50}Cr(III)$ stock solution of ca. 360 mg kg^{-1} was prepared by dissolution of the metal in a few millilitres of HCl followed by evaporation of excess HCl to about 1 mL and dilution with 1% HNO_3. An approximately 720 mg kg^{-1} solution of $^{53}Cr(VI)$ was prepared by dissolution of the metal oxide in a few millilitres of $HClO_4$ followed by evaporation of the contents to about 1 mL and addition of 10 mL DIW and 4.5 mL NH_4OH and dilution to 60 mL with DIW. Since no Cr(VI) species in the $^{50}Cr(III)$ stock and no Cr(III) species in the $^{53}Cr(VI)$ stock were detected, the concentrations of these species-specific spike solutions were quantified by reverse spike isotope dilution against the 112 mg kg^{-1} natural abundance Cr standard solution.

Sample preparation for determination of total Cr in yeast

Four 0.20-g sub-samples of yeast were accurately weighed into individual pre-cleaned Teflon digestion vessels. A suitable amount of the enriched ^{50}Cr spike was then added to each vessel to result in a ratio of measured isotopes which was close to unity. This could be achieved following workup of an initially unspiked sample so as to estimate the approximate concentration of the analyte. Three method blanks (spiked with 10% of the amount of enriched isotope solution used for the samples) were processed along with the samples. After 5 mL HNO_3 and 0.2 mL H_2O_2 were added, the vessels were capped and the contents digested in a CEM MDS-2100 microwave oven using the following heating conditions: 10 min at 20 psi and 40% power; 10 min at 40 psi and 50% power; 10 min at 80 psi and 50% power; 20 min at 100 psi and 60% power; 30 min at 120 psi and 70% power. After cooling, 0.25-mL volumes of the digested solutions were transferred to pre-cleaned polyethylene screw-capped bottles and diluted to 25 mL with 1% HNO_3. The samples were then diluted 20-fold prior to analysis by SF-ICP-MS. No dry weight correction was applied to the final results.

Calibration of the 350 mg kg^{-1} ^{50}Cr-enriched spike solution was achieved by reverse spike isotope dilution. This measurement was performed at the same time as the measurements of total Cr in the yeast digests. Four replicate samples were prepared by accurately weighing 0.1020 g of 350 mg kg^{-1} ^{50}Cr spike solution into pre-cleaned polyethylene screw-capped bottles. A 0.3930-g aliquot of the 112 mg kg^{-1} natural abundance Cr standard solution was added to each bottle. The contents of each bottle were then diluted with 15 mL 1% HNO_3. The samples were diluted 100-fold prior to analysis by SF-ICP-MS.

The digested yeast samples and the four reverse spike ID calibration samples were analysed by SF-ICP-MS under medium resolution on the same day. Mass bias correction was implemented, based on the theoretical natural abundance ratio of an isotope pair divided by the mean value of the isotope pair measured in a natural abundance Cr standard. The Element2 SF-ICP-MS was optimized daily by following recommendations of the manufacturer; operating conditions are summarized in Table 1.

Sample preparation for determination of Cr(III) and Cr(VI) in yeast

The extraction procedure used in this study followed that described by Kingston et al. [11–15, 17] with minor modification. An SSDSID MS approach was taken. Three sample blanks and three sub-samples of yeast were prepared at the same time. A 0.20-g sub-sample of yeast and 0.74 g Na_2CO_3 were weighed into a 100-mL Teflon beaker. After addition of 5 mL 2.5 M NaOH, 1.014 g 360 mg kg^{-1} $^{50}Cr(III)$ spike solution and 1.007 g 720 mg kg^{-1} $^{53}Cr(VI)$-enriched spike, DIW was added to the beaker to a total volume of 25 mL, resulting in concentrations of 0.5 M NaOH and 0.28 M Na_2CO_3. The beakers were then covered with lids and heated on a hot plate to maintain a solution temperature of 95±2 °C for 6 h. After cooling, the digests were transferred to pre-cleaned polyethylene screw-capped bottles and diluted to 30 g with DIW. Just before analysis, 0.5 g digest was diluted to 20 mL with DIW and the pH adjusted to 6 with HNO_3.

Calibration of the 360 mg kg^{-1} $^{50}Cr(III)$ and 720 mg kg^{-1} $^{53}Cr(VI)$ spike solutions was achieved by

Table 1 Experimental conditions

Parameter	Value
SF-ICP-MS	
Rf power	1,200 W
Plasma Ar gas flow rate	15.0 L min^{-1}
Auxiliary Ar gas flow rate	1.0 L min^{-1}
Nebulizer Ar gas flow rate	1.065 L min^{-1}
Sampler cone orifice (nickel)	1.00 mm
Skimmer cone orifice (nickel)	0.88 mm
Lens voltage	Focus:−826 V; x deflection: −1.20 V; y deflection: 1.40 V; shape: 105 V
Dead time	16 ns
Resolution	4,000
Data acquisition	E-scan, 800 passes, 100% mass window, 0.001-s settling time, 0.025-s sample time, 10 samples per peak
LC	
Column	Dionex, Omnipac PAX-500, 4.6 mm×250 mm
Eluent	0.06 M nitric acid/ammonium hydroxide solution at pH 7.0
Flow rate	1.00 mL min^{-1}
Injection volume	100 μL

reverse spike isotope dilution. Four replicate samples of each Cr species were separately prepared by accurately weighing a 0.1020 g of the 360 mg kg^{-1} ^{50}Cr (III) solution and 0.1000 g of the 720 mg kg^{-1} ^{53}Cr(VI) spike solution into pre-cleaned polyethylene screw-capped bottles. An aliquot of 0.3930 g and 1.220 g of the 112 mg kg^{-1} natural abundance Cr standard solution was added to the bottles containing ^{50}Cr (III) and ^{53}Cr (VI), respectively. The contents of each bottle were then diluted with 15 mL 1% HNO_3. The samples were further diluted 100-fold with 1% HNO_3 prior to analysis by SF-ICP-MS.

For quantitation of Cr(III) and Cr(VI), data acquisition on the Element2 was manually triggered following injection of the sample onto the LC column. Isotopes of ^{50}Cr, ^{52}Cr and ^{53}Cr were monitored during every run. A 100 ng g^{-1} solution of a natural abundance Cr standard containing both Cr(III) and Cr(VI) was used for mass bias correction, as described above. At the end of the chromatographic run, the acquired data were transferred to an off-line computer used to calculate peak areas of the Cr(III) and Cr(VI) transients and to generate ^{50}Cr/^{52}Cr (III), ^{53}Cr/^{52}Cr (III) ^{50}Cr/^{52}Cr (VI) and ^{53}Cr/^{52}Cr (VI) ratios, from which the analyte concentrations in the yeast were calculated by following the procedure reported earlier [11–13, 17]. Peak areas for Cr (III) and Cr(VI) were obtained by manually adjusting the integration limits to envelope the signals (integration limits of 90–250 and 350–360 s, respectively), while implementing background subtraction corrected for a sloped response.

Results and discussion

Total Cr in yeast

Isotope dilution and reverse isotope dilution permitted accurate quantitation of total Cr content in the yeast sample. The following equation was used for this purpose:

$$C = C_z \cdot \frac{m_y}{m_x} \cdot \frac{m_z}{m_y'} \cdot \frac{A_y - B_y \cdot R_n}{B_{xz} \cdot R_n - A_{xz}} \cdot \frac{B_{xz} \cdot R_n' - A_{xz}}{A_y - B_y \cdot R_n'} - C_b \quad (1)$$

where C is the blank corrected total concentration of Cr in the yeast (mg kg^{-1}); C_z is the concentration of primary assay Cr standard (mg kg^{-1}); m_y is the mass of spike used to prepare the blend solution of sample and spike (g); m_x is the mass of yeast sample used (g); m_z is the mass of primary assay Cr standard (g); m_y' is the mass of spike used to prepare the blend solution of spike and primary assay Cr standard solution (g); A_y is the abundance of the reference isotope in the spike; B_y is the abundance of the spike isotope in the spike; A_{xz} is the abundance of the reference isotope in the sample or primary assay standard; B_{xz} is the abundance of the spike isotope in the sample or primary assay standard; R_n is the measured reference/spike isotope ratio (mass-bias-corrected) in the blend solution of sample and spike; R_n' is the measured reference/spike isotope ratio (mass-bias-corrected) in the blend solution of spike and inorganic Cr standard; C_b is the analyte concentration in the blank (mg kg^{-1}) normalized to sample weight m_x.

Medium-resolution (MR) mode was used to resolve interferences from $^{38}Ar^{12}C^+$, $^{36}Ar^{14}N^+$, $^{40}Ar^{12}C^+$, $^{35}Cl^{16}O^1H^+$, $^{37}Cl^{16}O^+$ and $^{40}Ar^{12}C^+$ on the ^{50}Cr and ^{52}Cr isotopes. A mass-bias-corrected ratio for ^{52}Cr/^{50}Cr of 19.280±0.072 (one standard deviation, n=3) obtained by introduction of an unspiked yeast digest was not significantly different from the expected IUPAC [22] natural abundance ratio of 19.2832. This confirmed that no significant spectroscopic interferences on these isotopes arise from constituents in the sample matrix, permitting accurate results to be obtained using the selected isotope pair. A concentration of 2,014±16 mg kg^{-1} (one standard deviation, n=4) was obtained for the yeast. The calculated procedural detection limit for determination of total Cr was based on response from three ^{50}Cr-spiked blanks. A value of 0.3 mg kg^{-1} was estimated, based on three times the standard deviation of measured concentrations normalized to a 0.20-g sub-sample.

Quantitation of ^{50}Cr(III) and ^{53}Cr(VI) in enriched spike concentrations

In a preliminary study, individual enriched ^{50}Cr(III) and ^{53}Cr(VI) spike solutions were subjected to LC-SF-ICP-MS analysis. This permitted detection of each species in either

solution. Neither Cr (VI) species in the ^{50}Cr(III) stock solution nor Cr(III) species in the ^{53}Cr(VI) stock solution was detected. Thus, the concentrations of the enriched spike stocks could be quantified by a simple reverse spike isotope dilution against a natural abundance Cr standard without the need for an LC column separation. The following equation was used for this purpose:

$$C_y = \frac{C_z \cdot m_z}{m'_y} \cdot \frac{B_z \cdot R'_n - A_z}{A_y - B_y \cdot R'_n} \cdot \frac{AW_y}{AW_z} \quad (2)$$

where C_y is the concentration of ^{50}Cr(III) or ^{53}Cr(VI) in the enriched spikes (mg kg^{-1}); C_z is the concentration of natural abundance Cr standard (mg kg^{-1}); m_z is the mass of natural abundance Cr standard (g);m'_y is the mass of enriched spike used to prepare the blend solution of spike and natural abundance Cr standard solution (g); A_y is the abundance of the reference isotope in the spike; B_y is the abundance of the spike isotope in the spike; A_z is the abundance of the reference isotope in the natural abundance Cr standard; B_z is the abundance of the spike isotope in the natural abundance Cr standard; R'_n is the measured reference/spike isotope ratio (mass-bias-corrected) in the blend solution of spike and natural abundance Cr standard. Similar to measurements of total Cr, MR mode was used and concentrations of 365.6± 1.3 and 716.2±3.1 mg kg^{-1} (one standard deviation, n=4) were obtained for ^{50}Cr(III) and ^{53}Cr(VI) in the enriched spikes, respectively. These values were used for subsequent calculation of Cr(III) and Cr(VI) concentrations in the yeast sample.

Quantitation of Cr(III) and Cr(VI) in yeast

A Dionex anion exchange column, Omnipac PAX-500, was chosen for the separation of Cr(III) and Cr(VI). Initially, a 0.06 M nitric acid/ammonium hydroxide solution at pH 3 at a flow rate of 1.00 mL min^{-1} was used as eluent for species separation, similar to methods reported previously [11–15, 17]. Under these conditions, response from Cr(III) and Cr (VI) was almost baseline-resolved; however, broadening and peak tailing for Cr(VI) was noted. As higher eluent pH improved the Cr(VI) peak shape, the effect of this parameter was investigated. As expected, peak shape and retention time for Cr(VI) improved, whereas peak tailing by Cr(III) was observed as pH increased from 3 to 9. Retention time for Cr(III) increased from 80 s to 130 s as pH increased from 3 to 9. As a consequence, pH 7 was chosen for subsequent work as it provided a good compromise between resolution and peak shape for both Cr(III) (110-s retention time) and Cr(VI) (370-s retention time), as evident in Fig. 1.

Isotope dilution analysis is based on the principle that the added spike(s) are equilibrated with the endogenous

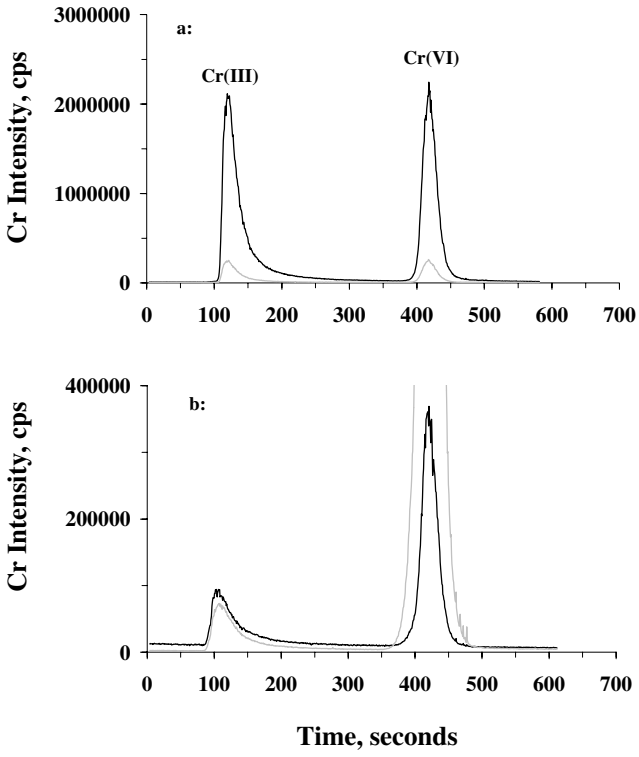

Fig. 1 LC SF-ICP-MS chromatograms: **a** 100 ng g^{-1} natural abundance Cr(III) and Cr(VI) standard; **b** spiked yeast extract; *black trace*^{52}Cr, *grey trace*^{53}Cr

forms of the analyte in the sample prior to ratio measurements. Under such circumstances, complete recovery efficiency is not needed. Results would be biased low if equilibration between the spike and the sample was not achieved (as the spike is typically recovered with greater efficiency during extraction). Accurate determination of the speciation of Cr in yeast samples using SSDSID MS calibration requires an extraction methodology which *releases* all analytes from the yeast matrix because the added spikes will not be metabolically incorporated into the yeast cells to subsequently equilibrate with the endogenous (protein)-bound Cr(III) and Cr(VI) species already present. Rather, the yeast cell wall must first be ruptured with the alkaline extraction medium, liberating the inorganic Cr(III) and Cr(VI) in the process. Thereafter, the opportunity to achieve equilibration with the added enriched spikes occurs in the aqueous phase. The resulting suspension presents a complex mixture for multi-phase chemical reactions, including redox and adsorption processes. Addition of the enriched spikes prior to digestion serves to subject the spike species to these processes, but it is clear that accurate results for quantitation of Cr(III) and Cr(VI) can only be achieved if complete liberation of all Cr from the yeast cells occurs. SSDSID MS provides an elegant approach to attaining this information and may be considered as the current state-of-the-art, with the exception of XAFS or

XANES, for which a synchrotron light source is needed to probe oxidation states directly in solids.

A 1-h alkaline extraction consisting of 0.28 M Na_2CO_3/0.5 M NaOH at 95±2 °C was initially applied to the yeast sample. This procedure had earlier been successfully used for extraction of Cr(VI) from solid environmental samples [11–15, 17]. To evaluate the extraction efficiency, yeast samples spiked with known amounts of natural abundance Cr(III) and Cr(VI) were analysed using the SSDSID MS method. Spike recovery was calculated as the difference between the measured analyte concentration in the spiked yeast sample and the analyte concentration in the unspiked sample divided by the spike concentration. Note that these recovery data do not reflect a conventional spike recovery test but rather an accuracy check which accounts for interconversions between Cr species and the low solubility of Cr(III) in an alkaline solution. Quantitative recovery of Cr(VI) and approximately 80% recovery of Cr(III) spikes from the yeast samples were obtained for both microwave and hot plate heating using SSDSID MS calibration. It is unlikely that the metabolically bound form of Cr(VI) in the yeast is more easily released to solution than that of the Cr(III) during the extraction. Thus, an apparent recovery of 100% Cr(VI) and only 80% Cr(III) following a 1-h extraction had to be attributed to other factors. It was noted that under the conditions utilized in these studies, spikes of Cr(III) produced visible precipitates, even in yeast-free solutions. Additionally, a much smaller Cr(III) peak was obtained by LC-SF-ICP-MS in such a solution compared to a similar concentration of Cr(III) in a standard, most likely a consequence of hydrolysis. Increased heating time served to dissolve this residue. This phenomenon was not evident for the Cr(VI) spikes. Thus, a longer heating time was required to aid equilibration processes and ensure accurate analysis of Cr(III). Since spike recoveries for either species were independent of the heating method, hot plate heating was chosen for subsequent experiments owing to its simplicity. Quantitative recoveries (100%) of both Cr (III) and Cr (VI) from yeast were subsequently obtained when a 6-h reflux at 95±2 °C was applied, as shown in Fig. 2. Thus, alkaline extraction using 0.28 M Na_2CO_3/0.5 M NaOH at 95±2 °C for 6 h was used for the final quantitation of Cr(III) and Cr (VI) in the yeast sample.

The following equations, reported previously [11–15, 17], were used for the quantitation of Cr(III) and Cr(VI) concentrations in the yeast samples:

$$R^{III}_{50/52} = \frac{\left({}^{50}A_x \cdot C^{III}_x \cdot W_x + {}^{50}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)(1-\alpha) + \left({}^{50}A_x \cdot C^{VI}_x \cdot W_x + {}^{50}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)\beta}{\left({}^{52}A_x \cdot C^{III}_x \cdot W_x + {}^{52}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x \cdot C^{VI}_x \cdot W_x + {}^{52}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)\beta} \quad (3)$$

$$R^{III}_{53/52} = \frac{\left({}^{53}A_x \cdot C^{III}_x \cdot W_x + {}^{53}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)(1-\alpha) + \left({}^{53}A_x \cdot C^{VI}_x \cdot W_x + {}^{53}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)\beta}{\left({}^{52}A_x \cdot C^{III}_x \cdot W_x + {}^{52}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)(1-\alpha) + \left({}^{52}A_x \cdot C^{VI}_x \cdot W_x + {}^{52}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)\beta} \quad (4)$$

$$R^{VI}_{50/52} = \frac{\left({}^{50}A_x \cdot C^{III}_x \cdot W_x + {}^{50}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)\alpha + \left({}^{50}A_x \cdot C^{VI}_x \cdot W_x + {}^{50}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)(1-\beta)}{\left({}^{52}A_x \cdot C^{III}_x \cdot W_x + {}^{52}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)\alpha + \left({}^{52}A_x \cdot C^{VI}_x \cdot W_x + {}^{52}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)(1-\beta)} \quad (5)$$

$$R^{VI}_{50/52} = \frac{\left({}^{53}A_x \cdot C^{III}_x \cdot W_x + {}^{53}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)\alpha + \left({}^{53}A_x \cdot C^{VI}_x \cdot W_x + {}^{53}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)(1-\beta)}{\left({}^{52}A_x \cdot C^{III}_x \cdot W_x + {}^{52}A^{III}_s \cdot C^{III}_s \cdot W^{III}_s\right)\alpha + \left({}^{52}A_x \cdot C^{VI}_x \cdot W_x + {}^{52}A^{VI}_s \cdot C^{VI}_s \cdot W^{VI}_s\right)(1-\beta)} \quad (6)$$

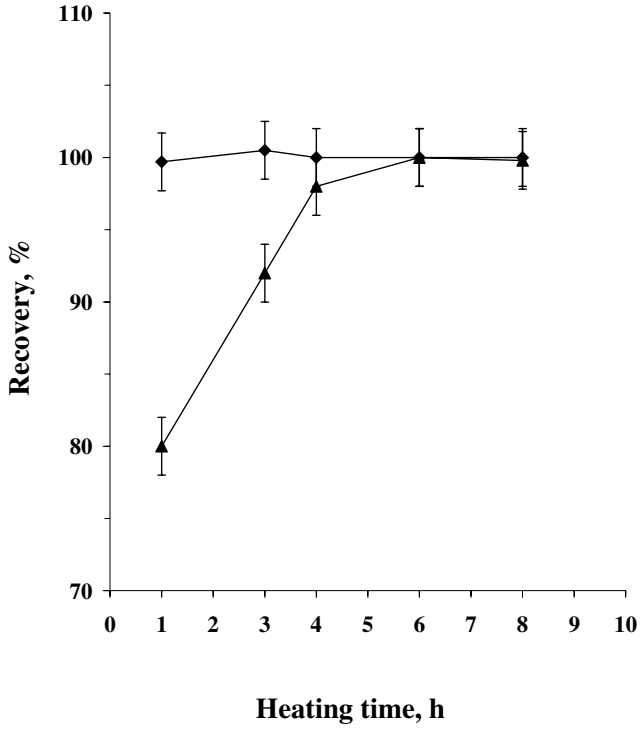

Fig. 2 Spike recovery versus heating time: ◆ Cr(VI), ▲ Cr(III)

where $R^{III}_{50/52}$, $R^{III}_{53/52}$, $R^{V}_{50/52}$ *and* $R^{VI}_{53/52}$ are measured isotope ratios of Cr(III) and Cr(VI) peaks in the spiked sample; ${}^{i=50,52,53}A_x$ is the abundance of isotope in the sample; ${}^{i=50,52,53}A_s$ is the abundance of isotope in the spikes; W_x is the sample mass (g); W^{III}_s is the mass of Cr(III) spike (g); W^{VI}_s is the mass of Cr(VI) spike (g); C^{III}_x is the concentration of Cr(III) in the sample (mg kg^{-1}, unknown); C^{VI}_x is the concentration of Cr(VI) in the sample (mg kg^{-1}, unknown); C^{III}_s is the concentration of Cr(III) in the Cr(III) enriched spike (mg kg^{-1}); C^{VI}_s is the concentration of Cr (VI) in the Cr(VI) enriched spike (mg kg^{-1}); α is the fraction of Cr(III) oxidized to Cr (VI) after spiking (unknown) and β is the fraction of Cr(VI) reduced to Cr (III) after spiking (unknown). Detailed information pertaining to the determination of C^{III}_x, C^{VI}_x, α *and* β is available in EPA method 6800 [17]. Concentrations of 1,952±103 and 76±48 mg kg^{-1} (one standard deviation, n=3) were obtained for Cr(III) and Cr(VI) in this yeast, respectively. Similar to previous reports for environmental samples [11], oxidation of Cr(III) to Cr(VI) was noted during alkaline extraction of this yeast sample (an α value of 24.2±7.6% was obtained, n=3). It was found that, even with alkaline extraction and chromatographic separation at pH 7, about 38% (a β value of 37.6±6.5%, n=3) of Cr(VI) was reduced to Cr(III) after spiking. This may be due to the yeast matrix containing potent reducing functional groups such as –SH, capable of reducing Cr(VI) to Cr(III) during the sample extraction.

Procedural detection limits for Cr(III) and Cr(VI) were calculated based on response from three spiked blank samples. Values of 30 and 2 mg kg^{-1} for Cr(III) and Cr (VI), respectively, were estimated, based on three times the standard deviation of measured concentrations normalized to a 0.20-g sub-sample.

Method validation

There is no biological material available with certified values for Cr(III) and Cr(VI) that can be used to verify the accuracy of this methodology. As a consequence, yeast samples fortified with known amounts of natural abundance Cr(III) and Cr(VI) were analysed. Results are summarized in Table 2. Measured concentrations of 3,749±43 and 2,466±40 mg kg^{-1} (n=3) for Cr(III) and Cr(VI), respectively, in the spiked yeast agree with the expected calculated concentrations of 3,736±103 and 2,475± 48 mg kg^{-1} (reflecting the sum of the added concentration and the analyte concentration derived from the yeast) for Cr (III) and Cr(VI), respectively. Despite significant bidirectional Cr species transformations noted during processing of this yeast sample (α and β values noted above), quantitative recoveries for both Cr(III) and Cr(VI) were obtained using this procedure, demonstrating the utility of the SSDSID MS methodology. This principle has also been successfully applied to the speciation of butyltin in sediments using multiple isotopically enriched butyltin spikes [23–26]. Furthermore, no significant difference between the sum of Cr(III) and Cr(VI) concentrations in the yeast and the total Cr concentration is evident. These observations confirm that the developed method is suitable for the accurate determination of Cr(III) and Cr(VI) in yeast.

Table 2 Results for speciation of Cr in yeast

Sample	^{Nat}Cr(III) added (mg kg^{-1})	^{Nat}Cr(VI) added (mg kg^{-1})	Measured Cr(III) (mg kg^{-1}, n=3)	Measured Cr(VI) (mg kg^{-1}, n=3)	^{Nat}Cr(III) recovery (%, n=3)	^{Nat}Cr(VI) recovery (%, n=3)	Measured Cr(III)+ Cr(VI) (mg kg^{-1}, n=3)	Measured total Cr (mg kg^{-1}, n=4)
Yeast	0	0	1,952±103	76±48	NA	NA	2,028±57	2,014±16
Spiked yeast	1,784	2,398	3,749±43	2,466±40	101±2	100±2	NA	NA

Conclusion

Despite significant bidirectional species transformations occurring during sample preparation, an accurate method was developed for the simultaneous determination of Cr (III) and Cr(VI) in yeast using SSDSID MS based on LC-SF-ICP-MS. The major form of Cr was found to be Cr(III), with Cr(VI) accounting for less than 5% of the total. Mass balance between the concentrations of total Cr and the sum of those for Cr(III) and Cr(VI) was achieved. The proposed method provides detection limits of 0.3, 2 and 30 mg kg^{-1} for total Cr, Cr(VI) and Cr(III), respectively, that are sufficiently low to make it well suited for the quantitation of these species not only in yeast supplements, but for monitoring Cr in dietary supplements in general.

References

1. Nriagu JO, Nieboer E (1988) Chromium in the natural and human environments. Wiley, New York
2. Anderson RA (1997) J Am Coll Nutr 16:404-410
3. Porter DJ, Raymond LW, Anastasio GD (1999) Arch Family Med 8:386–390
4. Shils ME, Olsen JA, Shike M (eds) (1999) Modern nutrition in health and disease, 9th edn. Williams and Wilkins, Baltimore, pp 277–282
5. Anderson RA (2000) Diabetes Metab 26:22–27
6. Anderson RA (1998) Nutr Rev 56:266–270
7. Campbell WW, Joseph LJ, Davey SL, Cyr-Campbell D, Anderson RA, Evans WJ (1999) J Appl Physiol 86:29–39
8. Gordon JB (1991) West J Med 154:352–355
9. Press RI, Geller J, Evans GW (1993) West J Med 152:41–45
10. Wang MM (1989) Nutr Res 9:989–998
11. Huo D, Kingston HM (2000) Anal Chem 72:5047–5054
12. Kingston HM, Huo D, Lu Y, Chalk S (1998) Spectrochim Acta Part B 53:299–309
13. Huo D, Lu Y, Kingston HM (1998) Environ Sci Technol 32:3418–3423
14. Kingston HM, Cain R, Huo D, Mizanur Rahman GM (2005) J Environ Monit 7:899–905
15. Mizanur Rahman GM, Kingston HM, Towns TG, Vitale RJ, Clay KR (2005) J Anal Bioanal Chem 382:1111–1120
16. Vitale RJ, Mussoline GR, Peura JC, James BR (1994) J Environ Qual 23:1249–1256
17. USEPA (2004) Method 6800, elemental and speciated isotope-dilution mass spectrometry, tests methods for evaluating solid waste, physical/chemical methods. SW 846, Update IVA. US Government Printing Office (GPO), Washington DC
18. Yang L, Mester Z, Abranko L, Sturgeon RE (2004) Anal Chem 76:3510–3516
19. Yang L, Mester Z, Sturgeon RE (2003) J Anal At Spectrom 18:1365–1370
20. Ding H, Olson LK, Caruso JA (1996) Spectrochim Acta Part B 51:1801–1812
21. Nelms SM, Quétel CR, Prohaska T, Vogl J, Tyalor PDP (2001) J Anal At Spectrom 16:333–338
22. Böhlke JK, de Laeter JR, De Bièvre P, Hidaka H, Peiser HS, Rosman KJR, Taylor PDP (2005) Isotopic compositions of the elements 2001. J Phys Chem Ref Data 34:57–67
23. Rodríguez-González P, Ruiz Encinar J, García Alonso JI, Sanz-Medel A (2003) Analyst 128:447–452
24. Centineo G, Rodríguez-González P, González EB, García Alonso JI, Sanz-Medel A (2004) Anal Bioanal Chem 381:380–387
25. Ruiz Encinar J, Rodríguez-González P, Rodríguez-Fernández J, JI García Alonso JI, Díez S, Bayona JM, and Sanz-Medel A (2002) Anal Chem 74:5237–5242
26. Ruiz Encinar J, Rodríguez-González P, García Alonso JI, Sanz-Medel A (2002) Anal Chem 74:270–281